THE UNIVERSITY OF WESTERN ONTARIO
BIOHAZARDOUS AGENTS REGISTRY FORM
Approved Biohazards Subcommittee: July 25, 2008
Biosafety Website: www.uwo.ca/humanresources/biosafety/

This form must be completed by each Principal Investigator holding a grant administered by the University of Western Ontario or in charge of a laboratory/facility where the use of Level 1, 2 or 3 biohazardous agents are described in the laboratory or animal work proposed. The form must also be completed if any work is proposed involving animals carrying zoonotic agents infectious to humans or involving plants, fungi, or insects that require Health Canada (HC) or Canadian Food Inspection Agency (CFIA) permits. The form must also be completed if any work is proposed involves plants or insects that require Health Canada (HC) or Canadian Food Inspection Agency (CFIA) permits.

This form must also be updated at least every 3 years or when there are changes to the biohazards being used.

Containment Levels will be required in accordance with Laboratory Biosafety Guidelines, 3rd edition, 2004, Health Canada (HC) or Containment Standards for Veterinary Facilities, 1st edition 1996, Canadian Food Inspection Agency (CFIA).

Completed forms are to be returned to Occupational Health and Safety, OHS (Stevenson-Lawson Building, Room 295) for distribution to the Biohazard Subcommittee. For questions regarding this form, please contact the Biosafety Officer at extension 81135. If there are changes to the information on this form (excluding grant title and funding agencies), modifications must be submitted to Occupational Health and Safety. See website: www.uwo.ca/humanresources/biosafety/

PRINCIPAL INVESTIGATOR Edmund MK Lui
SIGNATURE
DEPARTMENT Phy & Pharm
ADDRESS M288
PHONE NUMBER 83320
EMAIL ed.lui@schulich.uwo.ca

Location of experimental work to be carried out: Building(s) MSB and DSB Room(s) MSB-521 & 496; DSB 2003

*For work being performed at Institutions affiliated with the University of Western Ontario, the Safety Officer for the Institution where experiments will take place must sign the form prior to its being sent to Occupational Health and Safety (See Section 12.0, Approvals). For research being done at Lawson Health Research Institute, London Regional Cancer Program, Child and Parent Research Institute, or Robarts Research Institute, a University Biosafety Committee member can also sign as the Safety Officer for the Institution.

FUNDING AGENCY/AGENCIES: Ontario Rsearch Fund, Ministry of Research and Innovation
GRANT TITLE(S): New technologies for ginseng agriculture and product development

PLEASE ATTACH A BRIEF DESCRIPTION OF YOUR WORK THAT EXPLAINS THE BIOHAZARDS USED AND HOW THEY WILL BE USED. PROJECTS SUBMITTED WITHOUT A SUMMARY WILL NOT BE REVIEWED.

Medicinal herbal products generally contain multiple components and a spectrum of pharmacological activities. Generally these products have long history of cultural use and are considered as safe; although occasionally toxicity has been reported due to contamination or adulterated products. We will focus on the anti-oxidative, immunomodulation, vascular protective and anti-inflammatory effects of ginsengs and other Chinese medicinal herbs for the treatment of cancer, inflammation as well as metabolic and vascular diseases. We use state-of-the- art methodology to i) elucidate their pharmacological mechansim of action; ii) correlate biological activity

with chemical profile; and iii) isolate and identify pharmacologically active components. All of the herbal medicines to be tested have a long history of cultural use and have been subjected to scientific investigations. We use different cell lines (RAW 264.7, EA hy 926,B16, B16F10 melanoma cells) in mono-layers to test the pharmacological activities in vitro of herbal medicines: such as anti-oxidative, immuno-modulatory, anti-angiognesis and apoptosis effects. For immuno-modulation study, LPS is used as a positive control and is also used to induce inflammation to screen for anti-inflammatory activity.

In addition to in vitro experiments, in vivo animal experiments are used to validate in vitro findings. The following is a brief protocl for the in vivo experiments.

Part I. To determine the protective effect against Melanoma cell-induced liver metastasis, B16F10 melanoma cells will be injected to adult C57bl mice (Day 0), which will be examined for tumor yield in the liver 21 day later. For each extract or fraction three experimental groups (Low dose, Medium dose, and High dose of the herbal medicines) and I control (saline) will be required. (3 dose levels are usually required to define a dose-response relationship). From our experience with variability in the induction of liver metastasis, a N value of 8-10 is required.

Herbal medicine treatment protocol:

1. Standard: One day after melanoma cell injection and continues daily (except week end) for 20 days.
2. Pretreatment. Same as in 1 except with a pretreatment for 3 days before melanoma cell injection. This pretreatment protocol is to determine whether activation of immune function (more specifically Kupffer's cells) would enhance the protective effect. This could be done at one dose level for comparison with the standard treatment protocol.

Part I a. Estimated animals required per test extract by the standard protocol at 3 dose levels: 45 animals.
Part I b. Estimated animals required per test extract by the pretreatment protocol at one dose level: 35 animals.
Part II. To evaluate treatment effect on microvascular function in liver tumor and non-tumor sites by Intravital video microscopy (IVVM), 3 animals per treatment groups will be required.
Part III. To evaluate treatment effect on Kupffer cell function, mice will be pretreated with herbal product fractions or saline for 3 days prior to melanoma cell injection and monitored for 5 hrs (migration rate and interaction with melanoma cells in liver) by IVVM. Two dose levels that have previously been established in the metastasis model will be used. N=5

Part 2. To determine the protective effect of ginseng against homocysteine-induced vascular injury, 3-week-old Sprague-Dawley rats (40–50 g) will be used. The normal control group (n=6) will have free access to standard tap water, while the treatment group (n=6) will receive homocysteine in tap water (50 mg/kg body weight per d) for 42 d to induce vascular injury. To evaluate the protective effect, two groups will be given 500mg/kg and 1000 mg/kg of different ginseng extracts (aqueous and organic ones) by gastric gavage daily. Body weight will be recorded daily. Rats are killed at the end of the experiment and blood taken to prepare serum for quantitation of cytokines and oxidant stress markers. Liver will be taken for the examination of tissue cytokines and oxidant stress. The aorta will be removed for histological and organ bath studies to assess contractile-relaxation response. (This protocol will be reinstated from a previous one (2003-101-11) by a modification of the current protocol: 2008-02-01) protocol.

Names of all personnel working under Principal Investigators supervision in this location:

1) Jirui Hou (PDF)
2) Chike Azike (GS)
3) Hau Pei (Staff)
4) Yuan Liu (Visiting scholar)

1.0 Microorganisms

1.1 Does your work involve the use of microorganisms or biological agents of plant or animal origin (including but not limited to viruses, prions, parasites, bacteria)? O YES X NO

If no, please proceed to Section 2.0

Do you use microorganisms that require a permit from the CFIA? O YES O NO

If YES, please give the name of the species. _____

What is the origin of the microorganism(s)? _____

Please describe the risk (if any) of escape and how this will be mitigated:

_____

_____

_____

Please attach the CFIA permit.

Please describe any CFIA permit conditions:

_____

_____

_____

1.2 Please complete the table below:

Name of Biological agent(s)	Is it known to be a human pathogen? YES/NO	Is it known to be an animal pathogen? YES/NO	Is it known to be a zoonotic agent? YES/NO	Maximum quantity to be cultured at one time?

*Please attach a Material Safety Data Sheet or equivalent from the supplier.

2.0 Cell Culture

2.1 Does your work involve the use of cell cultures? **X YES** O NO

If no, please proceed to Section 3.0

2.2 Please indicate the type of primary cells (i.e. derived from fresh tissue) that will be grown in culture in the table below

Cell Type	Is this cell type used in your work?	Source of Primary Cell Culture Tissue
Macrophage	Yes	Mouse abdominal macrohgage
Lymphocyte	Yes	Spleen of mouse
Kupffer's cell	Yes	Liver of mouse

*** DESCRIPTION MUST BE ATTACHED TO THIS FORM OR PROJECT WILL NOT BE REVIEWED***

2.3 Please indicate the type of established cells that will be grown in culture in the table below.

Cell Type	Is this cell type used in your work?	Specific cell line(s)	Supplier / Source
Vascular endothelial cells	Yes	EA.hy 926	Dr. Edgell from the Univeristy of North Carolina
Melanoma cells	Yes	B16 F10 melanoma cells	Dr. Anne Chambers (London Regional Cancer Centre). It was tested negative for murine pathogens on Oct 25, 2005 by MU Research Animal Diagnostic Lab. Columbia MO. See attached report.
Macrophage	Yes	mouse RAW264.7	Dr. Jeff Dixon (Physiology & Pharmacology University of Western Ontario)
Other (specify)	No .		

*Please attach a Material Safety Data Sheet or equivalent from the supplier. (For more information, see www.atcc.org)

2.4 For above named cell types(s) indicate HC or CFIA containment level required OX 1 O 2 O 3

3.0 Use of Human Source Materials

3.1 Does your work involve the use of human source materials? O YES OX NO
If no, please proceed to Section 4.0

3.2 Indicate in the table below the Human Source Material to be used.

Human Source Material	**Source/Supplier /Company Name**	**Is Human Source Material Known to Be Infected With An Infectious Agent? YES/NO**	**Name of Infectious Agent (If applicable)**	**HC or CFIA Containment Level (Select one)**
Human Blood (whole) or other Body Fluid		O Yes O No		O 1 O 2 O 3
Human Blood (fraction) or other Body Fluid		O Yes O No		O 1 O 2 O 3
Human Organs or Tissues (unpreserved)		O Yes O No		O 1 O 2 O 3
Human Organs or Tissues (preserved)		O Yes O No		O 1 O 2 O 3

4.0 Genetically Modified Organisms and Cell lines

4.1 Will genetic modifications be made to the microorganisms, biological agents, or cells described in Sections 1.0 and 2.0? O YES **X NO** If no, please proceed to Section 5.0

4.2 Will genetic modification(s) involving plasmids be done? O YES, complete table below O NOBacteria Used for Cloning *	Plasmid(s) *	Source of Plasmid	Gene Transfected	Describe the change that results

* Please attach a Material Data Sheet or equivalent if available.

*** DESCRIPTION MUST BE ATTACHED TO THIS FORM OR PROJECT WILL NOT BE REVIEWED***

4.3 Will genetic modification(s) involving viral vectors be done? O YES, complete table below O NO

Virus Used for Transduction *	Vector(s) *	Source of Vector	Gene Transfected	Describe the change that results

* Please attach a Material Safety Data Sheet or equivalent.

4.4 Will genetic sequences from the following be involved?

- ♦ HIV O YES, please specify _____ O NO
- ♦ HTLV 1 or 2 or genes from any Level 1 or Level 2 pathogens O YES, specify _____ O NO
- ♦ SV 40 Large T antigen O YES O NO
- ♦ E1A oncogene O YES O NO
- ♦ Known oncogenes O YES, please specify _____ O NO
- ♦ Other human or animal pathogen and or their toxins O YES, please specify _____ O NO

4.5 Will virus be replication defective? O YES O NO

4.6 Will virus be infectious to humans or animals? O YES O NO

4.7 Will this be expected to increase the containment level required? O YES O NO

5.0 Human Gene Therapy Trials

5.1 Will human clinical trials be conducted using the viral vector in 4.0? O YES **X NO**
If no, please proceed to Section 6.0 If YES attach a full description of the make-up of the virus.

5.2 Will virus be able to replicate in the host? O YES O NO

5.3 How will the virus be administered? _____

5.4 Please give the Health Care Facility where the clinical trial will be conducted:_____

5.5 Has human ethics approval been obtained? O YES, number: _____ O NO O PENDING

6.0 Animal Experiments

6.1 Will live animals be used? **X YES** O NO If no, please proceed to section 7.0

6.2 Name of animal species to be used_ MOUSE & RAT

6.3 AUS protocol # 2008-018

6.4 Will any of the agents listed be used in live animals **X YES**, specify: Tested negative for murine pathogens on Oct 25, 2005 by MU Research Animal Diagnostic Lab. Columbia MO. O NO

*** DESCRIPTION MUST BE ATTACHED TO THIS FORM OR PROJECT WILL NOT BE REVIEWED***

7.0 Use of Animal species with Zoonotic Hazards

7.1 Will any of the following animals or their organs, tissues, lavages or other body fluids including blood be used?

♦ Pound source dogs	O YES	**X NO**
♦ Pound source cats	O YES	**X NO**
♦ Cattle, sheep or goats	O YES	**X NO**
♦ Non- Human Primates	O YES, please specify species _____	**X NO**
♦ Wild caught animals	O YES, please specify species & colony # _____	**X NO**
♦ Birds	O YES	**X NO**
♦ Others (wild or domestic)	O YES, please specify _____	**X NO**

8.0 Biological Toxins

8.1 Will toxins of biological origin be used? O YES **X NO** If no, please proceed to Section 9.0
(LPS will be used in cell culture as a positive control to induce inflammatory response at 1 ug/ml concentration. The risk for injury to researcher is minimal).
8.2 If YES, please name the toxin(s)_____
Please attach information, such as a Material Safety Data Sheet, for the toxin(s) used.

8.3 What is the LD_{50} (specify species) of the toxin_____

9.0 Insects Requiring CFIA Permits

9.1 Do you use insects that require a permit from the CFIA? O YES **X NO**
If no, please proceed to Section 10.0

9.2 If YES, please give the name of the species. _____

9.3 What is the origin of the insect? _____

9.4 What is the lifestage of the insect? _____

9.5 What is your intention? O Initiate and maintain colony, give location: _____
O "One-off" use, give location: _____

9.6 Please describe the risk (if any) of escape and how this will be mitigated:
_____
_____
_____

9.7 Please attach the CFIA permit.

9.8 Please describe any CFIA permit conditions:
_____
_____
_____

*** DESCRIPTION MUST BE ATTACHED TO THIS FORM OR PROJECT WILL NOT BE REVIEWED***

10.0 Plants Requiring CFIA Permits

10.1 Do you use plants that require a permit from the CFIA? O YES XO NO
If no, please proceed to Section 11.0

10.2 If YES, please give the name of the species. _____

10.3 What is the origin of the plant? _____

10.4 What is the form of the plant (seed, seedling, plant, tree…)? _____

10.5 What is your intention? O Grow and maintain a crop O "One-time" use

10.6 Do you do any modifications to the plant? O YES O NO
If yes, please describe: _____

10.7 Please describe the risk (if any) of loss of the material from the lab and how this will be mitigated:
_____
_____
_____

10.8 Is the CFIA permit attached? O YES O NO

10.9 Please describe any CFIA permit conditions:
_____
_____
_____

11.0 Import Requirements

11.1 Will the agent be imported? O YES, please give country of origin_____ XO NO
If no, please proceed to Section 10.0

11.2 Has an Import Permit been obtained from HC for human pathogens? O YES O NO

11.3 Has an import permit been obtained from CFIA for animal pathogens? O YES O _____NO

11.4 Has the import permit been sent to OHS? O YES, please provide permit # _____ O NO

12.0 Training Requirements for Personnel Named on Form

All personnel named on the above form who will be using any of the above named agents are required to attend the following training courses given by OHS:
- ♦ Biosafety
- ♦ Laboratory and Environmental/Waste Management Safety
- ♦ WHMIS (Western or equivalent)
- ♦ Employee Health and Safety Orientation

As the Principal Investigator, I have ensured that all of the personnel named on the form who will be using any of the biohazardous agents in Sections 1.0 to 9.0 have been trained.

SIGNATURE _____

*** DESCRIPTION MUST BE ATTACHED TO THIS FORM OR PROJECT WILL NOT BE REVIEWED***

13.0 Containment Levels

11.1 For the work described in sections 1.0 to 9.0, please indicate the highest HC or CFIA Containment Level required. XO 1 2 O 3

13.2 Has the facility been certified by OHS for this level of containment?

O YES, permit # if on-campus_____
O NO
O NOT REQUIRED

14.0 Procedures to be Followed

14.1 As the Principal Investigator, I will ensure that this project will follow the Western Biosafety Guidelines and Procedures Manual for Containment Level 1 & 2 Laboratories (and the Level 3 Facilities Manual for Level 3 projects). I will ensure that UWO faculty, staff and students have an up-to-date Position Hazard Communication Form, found at http://www.wph.uwo.ca/

SIGNATURE _____ Date:April 25, 2009_____

15.0 Approvals

UWO Biohazard Subcommittee: SIGNATURE: _____
Date:_____

Safety Officer for Institution where experiments will take place: SIGNATURE:_____
Date:_____

Safety Officer for University of Western Ontario (if different from above):SIGNATURE:_____
Date:_____

Approval Number: _____ Expiry Date (3 years from Approval): _____

Special Conditions of Approval:

DESCRIPTION MUST BE ATTACHED TO THIS FORM OR PROJECT WILL NOT BE REVIEWED*

Hi Jennifer,
The B16F10 cell line has not been modified.
EA.hy926 cells are a hybrid of HUVEC and a human lung carcinoma cell line A549 (Edgell et al., 1983. Proc Natl Acad Sci 80: 3734-3737). This cell line has been used by several groups
to carry out Matrigel-based tubulization of endothelial cells.
I hope this is what you need for documentation.
Thanks
Ed

>>> Jennifer Stanley <jstanle2@uwo.ca> 05/05/2009 2:29 pm >>>
Hi Dr. Lui
One more question - The B16F10 cell line was likely originally purchased
from ATCC (www.atcc.org) - do you know if the cell line has been modified by the Chambers lab in anyway? If so, please send the details on any genetic modifications that have been done...
Thanks,
Jennifer

Jennifer Stanley wrote:
> Hello Dr. Lui
> I received your form -thanks! I have one question, is the "EA.hy 926"
> cell line a rodent cell line or a human cell line? Do you have a
> website or something on these cells?
> Thanks
> Jennifer
>

-------- Original Message --------
Subject: Re: Cell line: EA.hy926
Date: Tue, 14 Jul 2009 14:46:52 -0400
From: Geneviève Lacroix <genevieve_lacroix@phac-aspc.gc.ca>
To: Jennifer Stanley <jstanle2@uwo.ca>

Dear Jennifer,

Again the same process applies, if this cell line has not been transformed
with a human pathogen or if it does not contain human pathogen, than we can consider it a risk group 1. As far as I can judge with the provided information, I would classify it as a RG1 but again, you should perform a
risk assessment to determine the risk group.

Regards

Genevieve Lacroix, M.Sc.
A/Head, Importation and Biosafety Programs
Chef intérimaire/Importation et servuce de biosécurité
Office of Laboratory Security / Bureau de la sécurité des laboratoires
Public Health Agency of Canada / Agence de la santé publique du Canada
100 ch. Colonnade Rd. AL: 6201A, Ottawa, Ontario, Canada, K1A 0K9
Tel: (613) 946-6982
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genevieve_lacroix@phac-aspc.gc.ca
http://www.phac-aspc.gc.ca/ols-bsl/index.html

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Cell Biology

ATCC® Number: CRL-2873™ Order this Item

Designations: HUVEC-CS

Biosafety Level: 1

Medium & Serum: See Propagation

Organism: *Homo sapiens* (human)

Price: $569.00

Depositors: IM Bird

Shipped: frozen

Growth Properties: adherent

Morphology: endothelial

PHOTO

Source:
Organ: umbilical vein
Tissue: vascular endothelium
Disease: normal
Cell Type: endothelial

Permits/Forms: In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.

Related Cell Culture Products

Isolation: **Isolation date:** August, 1998

Age: newborn

Comments: HUVEC-CS was derived from HUV-EC-C (ATCC CRL-1730?) by initially passaging in tissue culture plastic flasks without additional growth factor like ECGS. Subsequently the HUVEC-CS cell line was plated on gelatin-coated flasks. It exhibits positive acetylated low-density lipoprotein (AcLDL) uptake and expresses eNOS, CD31 and ve-cadherin (classical markers of endothelial cells).It spontaneously forms capillary-like structures when grown on Matrigel. Receptors were detected for angiotensin II (AII), bradykinin and ATP.[PubMed: 15350190]. Distribution freeze lots have a doubling potential of 5 to 8 PDLs.

Propagation: **ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated Dulbecco's Modified Eagle's Medium, Catalog No. 30-2002. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 20%.
Temperature: 37.0°C
Growth Conditions: Use gelatin-coated flasks.

Subculturing: **Protocol:** Volumes used in this protocol are for 75cm2 flask; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes.1. Remove and discard culture medium.2. Briefly rinse the cell layer with 0.05% (w/v) Trypsin- 0.53 mM EDTA solution (GIBCO Cat# 25300-054) to remove all traces of serum that contains trypsin inhibitor.3. Add 1.0 to 2.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes).Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting. To remove trypsin-EDTA solution, transfer cell suspension to a centrifuge tube and spin at approximately 125 xg for 5 to10 minutesDiscard supernatant and resuspend cells in fresh growth medium. Add appropriate aliquots of the cell suspension to new porcine gelatin coated culture vessels. An inoculum of 1X 10(4) to 2X 10(4) viable cells/cm2 is recommended. Place culture vessels in incubators at 37°C.
Subcultivation Ratio: 1: 3 to 1: 4

Preservation: **Freeze medium:** Culture medium,90%; DMSO, 10%
Storage temperature: liquid nitrogen vapor phase

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Cell Biology

ATCC® Number: CRL-6475™ Order this Item

Price: $264.00

Designations: B16-F10

Biosafety Level: 1

Shipped: frozen

Medium & Serum: See Propagation

Growth Properties: adherent

Organism: *Mus musculus* (mouse)

Morphology: melanocyte

PHOTO

Source:
Organ: skin
Strain: C57BL/6J
Disease: melanoma

Permits/Forms: In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.

Related Cell Culture Products

Applications: transfection host (technology from amaxa)

Propagation:
ATCC complete growth medium: The base medium for this cell line is ATCC-formulated Dulbecco's Modified Eagle's Medium, Catalog No. 30-2002. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%.
Temperature: 37.0°C
Atmosphere: air, 95%; carbon dioxide (CO_2), 5%

Subculturing:
Protocol:

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin- 0.53 mM EDTA solution to remove all traces of serum that contains trypsin inhibitor.
3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes).
   Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.
4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting.
5. Add appropriate aliquots of the cell suspension to new culture vessels.
6. Incubate cultures at 37°C.

Subcultivation Ratio: A subcultivation ratio of 1:10 is recommended
Medium Renewal: Every 2 to 3 days

Preservation:
Freeze medium: culture medium, 95%; DMSO, 5%
Storage temperature: liquid nitrogen vapor phase

Related Products: Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2002
recommended serum:ATCC 30-2020

References:
22151: Fidler IJ. Biological behavior of malignant melanoma cells correlated to their survival in vivo. Cancer Res. 35: 218-224, 1975. PubMed: 1109790
22191: Fidler IJ, et al. Tumoricidal properties of mouse macrophages activated with mediators from rat

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Cell Biology

ATCC® Number: CCL-185™ Order this Item

Designations: A549

Biosafety Level: 1

Medium & Serum: See Propagation

Organism: *Homo sapiens* (human)

Price: $256.00

Depositors: M Lieber

Shipped: frozen

Growth Properties: adherent

Morphology: epithelial

PHOTO

Source: **Organ:** lung
Disease: carcinoma

Cellular Products: keratin

Permits/Forms: In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.

Related Cell Culture Products

Isolation: **Isolation date:** 1972

Applications: transfection host (Nucleofection technology from Lonza
Roche FuGENE® Transfection Reagents)

Reverse Transcript: negative

DNA Profile (STR):
Amelogenin: X,Y
CSF1PO: 10,12
D13S317: 11
D16S539: 11,12
D5S818: 11
D7S820: 8,11
THO1: 8,9.3
TPOX: 8,11
vWA: 14

Cytogenetic Analysis: This is a hypotriploid human cell line with the modal chromosome number of 66, occurring in 24% of cells. Cells with 64 (22%), 65, and 67 chromosome counts also occurred at relatively high frequencies; the rate with higher ploidies was low at 0.4%. There were 6 markers present in single copies in all cells. They include der(6)t(1;6) (q11;q27); ?del(6) (p23); del(11) (q21), del(2) (q11), M4 and M5. Most cells had two X and two Y chromosomes. However, one or both Y chromosomes were lost in 40% of 50 cells analyzed. Chromosomes N2 and N6 had single copies per cell; and N12 and N17 usually had 4 copies.

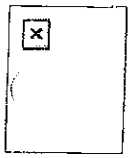
Isoenzymes: G6PD, B

Age: 58 years

Gender: male

Ethnicity: Caucasian

Comments: This line was initiated in 1972 by D.J. Giard, et al. through explant culture of lung carcinomatous tissue from a



FINAL REPORT OF LABORATORY EXAMINATION
MU Research Animal Diagnostic Laboratory
1600 East Rollins, Columbia MO 65211 1-800-669-0825 1-573-882-5983
radil@missouri.edu www.radil.missouri.edu

CASE NUMBER: 8053-2005

RECEIVED ON: 10/19/2005
COMPLETED ON: 10/25/2005

SUBMITTED BY:

Dave Dales
London Regional Cancer-Programme
790 Commissioners Rd. E.
London, Ontario
N6A 4L6
Canada
(519) 685-8600 x53271
[519] 685=8646 (fax)

SPECIMEN DESCRIPTION:

SPECIES: mouse
DESCRIPTION: cells
NUMBER OF SPECIMENS: 11

PURCHASE ORDER #: CCE-117285

ID

F1 B16F1
B16F10
D2A1
D2OR
PAP2
4T1
66cl4
168 FARN
67 NR
MDA MB 468 LN
MDA MB 435 HAL

PROFILE/EXAM REQUESTED: IMPACT III PCR Profile

SUMMARY: No pathogens were detected by PCR assays.

If you have questions, please call our toll free number at 1-800-669-0825 or e-mail us at radil@missouri.edu.